High-Resolution Mapping of the Spatial Organization of a Bacterial Chromosome

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Background

❑Hi-C.

❑For eukaryotes,deep sequencing, has enabled higher-resolution studies of chromosome structure in vivo.

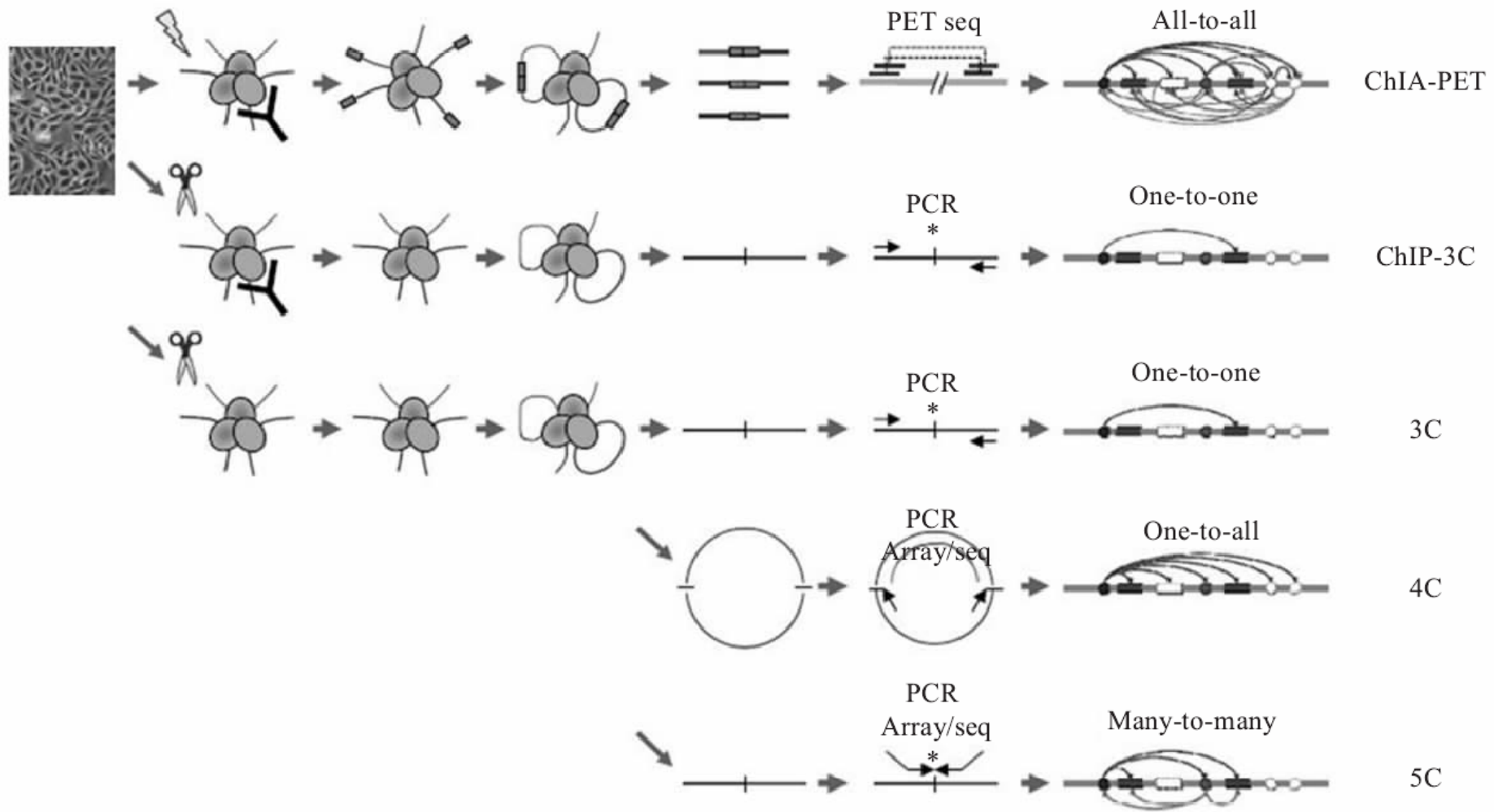❑TADs<1Mb in size, but the factors that create, maintain, and influence these domains are presently unknown.

Fig. 1　Schematic representation of ChIP-PET, ChIP-3C, 3C, 4C or 5C methods[2]

Abstract

❑ We used Hi-C to map the structure of bacterial chromosomes.

❑ The Caulobacter crescentus chromosome consists of CIDs.

❑ We provide evidence that domain boundaries are established by highly expressed genes and the formation of PFRs.

❑ How the histone-like protein HU and SMC promote affect on the structura of chromosome.

Experiment and Result

1. We performed Hi-C on swarmer cells .
2. We divided the genome into 10-kilobase (kb) bins,with interaction frequencies .
3. We visualized interactions as a heat map where each matrix position, m_{ij},reflects the relative frequency of interactions .
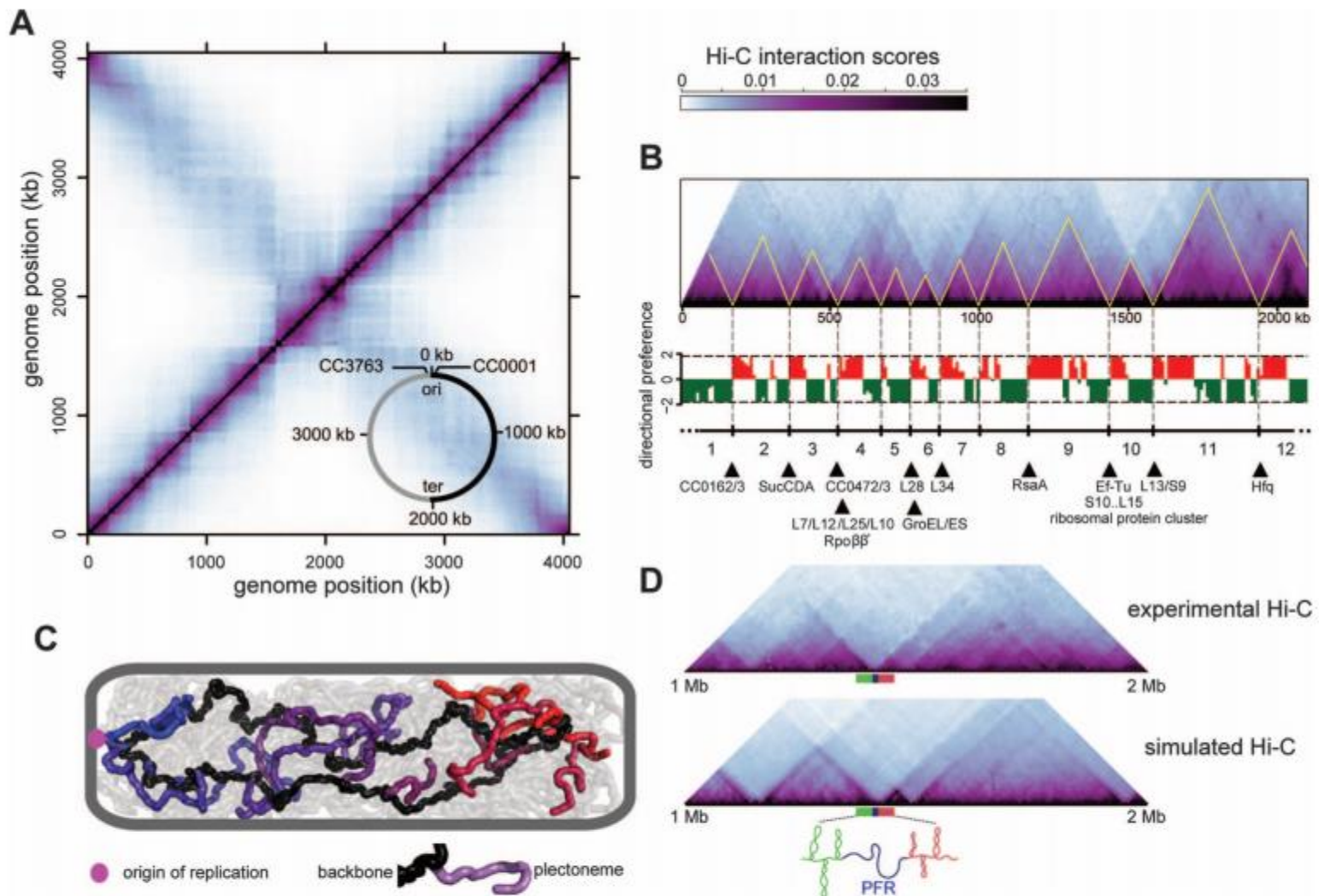
Fig. 1. Partitioning of the *Caulobacter* chromosome into CIDs. (A) Normalized NcoI Hi-C contact map for *Caulobacter* swarmer cells displaying contact frequencies for pairs of 10-kb bins across the genome. Axes indicate the genome position of each bin. (Inset) Simplified genomic map showing the origin of replication (*ori*) and terminus (*ter*), along with the right (black) and left (gray) chromosomal arms. (B) Hi-C contact map for one arm of the chromosome rotated 45° clockwise with directional preference plots below. Left-ward and rightward preferences are shown as green and red bars, respectively. CIDs are outlined in yellow and numbered. Highly expressed genes at CID boundaries are listed (hypothetical genes are designated by GenBank ID no.). (C) Polymer chromosome model showing the polarly anchored origin (magenta), chromosome backbone (black), and plectonemes (gray, with every 10th plectoneme on one arm in a color). (D) Comparison of experimental and simulated Hi-C contact maps, indicating that PFRs can account for CIDs.

- The main diagonal reflects high-frequency interactions between loci on the same chromosomal arm. The other less prominent diagonal captures lower-frequency inter-arm contacts.

- Loci within a CID interact preferentially with other loci within the same CID as compared to other CIDs.

- Loci at the border of each CID strongly favor interactions with loci on their left-or righthand side, but not both.

- The hierarchical organization resembles the so-called TADs.

- There were 23 CIDs, ranging in lengthfrom 30 to 420 kb .

- Of the 23 CID boundaries, 17 contained one or more highly transcribed genes.

- We hypothesized that high gene expression unwinds the DNA duplex and creates PFRs,which form barriers between CIDs.

Part 2 Modeling

1. The chromosome was modeled as a circular polymer comprising of plectonemes that have no sequence specificity and are stochastic in length and location.

2. We simulated the Hi-C procedure on 25,000 modeled chromosomes, and compared the resulting data to experimental Hi-C data.

3. By varying model parameters, we identified values that provided the best fit to the observed Hi-C contact frequencies.
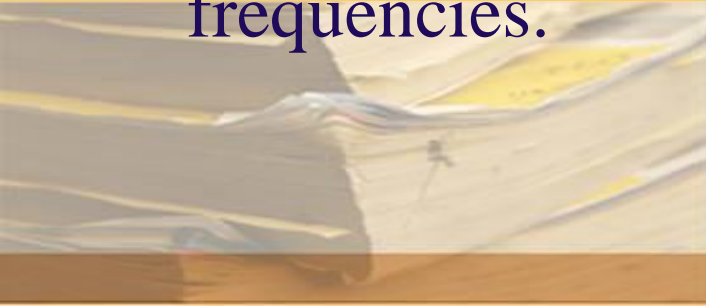
two broad levels of chromosomal organization.

❑ The DNA is arranged into a fiber of ~300 plectonemes separated by small spacers, resembling a bottle brush.

❑ The bottle brush fiber forms a circular chromosome tethered at the pole by an origin-proximal region with chromosomal arms in close proximity down the long axis of the cell.

❑ Simulated Hi-C data generated a pattern of CIDs that resembled those observed experimentally, supporting the hypothesis that PFRs can induce CIDs .
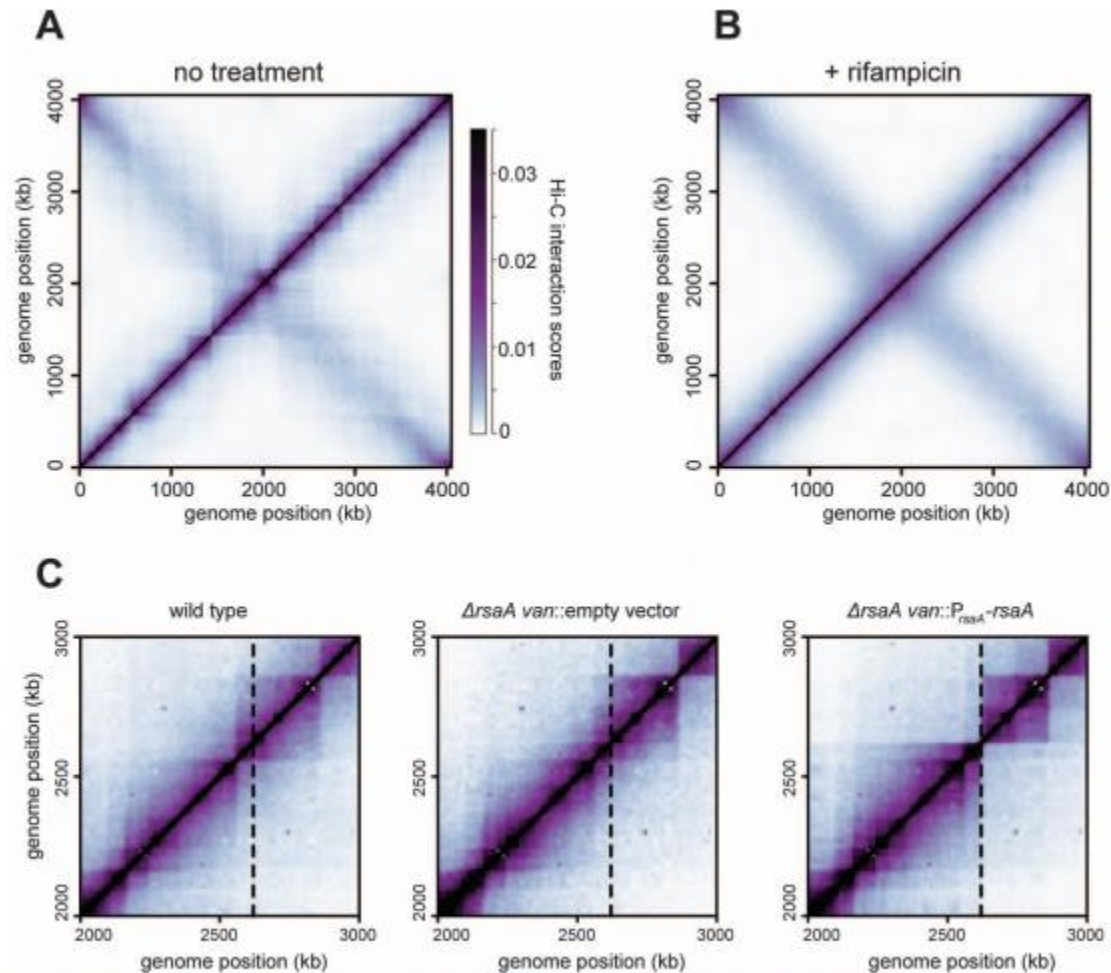
Part 3 The role of gene expression

Fig. 2. Effect of inhibiting transcription on CID boundaries. Normalized BglII Hi-C contact maps for (**A**) untreated and (**B**) rif-treated swarmer cells. (**C**) Hi-C contact maps for wild-type, $\Delta rsaA$, and $\Delta rsaA$ + van::P_{rsaA}-$rsaA$ cells. Only the region of the genome containing the van locus (dashed line) is shown.

rif-treated

❑ The interaction matrix of rif-treated cells was globally similar to that of untreated cells.

❑ CID boundaries were severely disrupted in rif-treated cells, Simulations of rif-treated chromosomes, performed by removing PFRs, also produced domain-free contact maps.

rsaA to the vanA

❑The insertion of rsaA generated a sharp new CID boundary.

❑Relocating rsaA to the xylX locus,also created a new CID boundary at this location.

❑We conclude that highly expressed genes play a direct role in defining chromosomal domain boundaries.

Novobiocin significantly reduced the frequency of interactions in the 20- to 200-kb range while modestly increasing interactions in the 200- to 800-kb range .
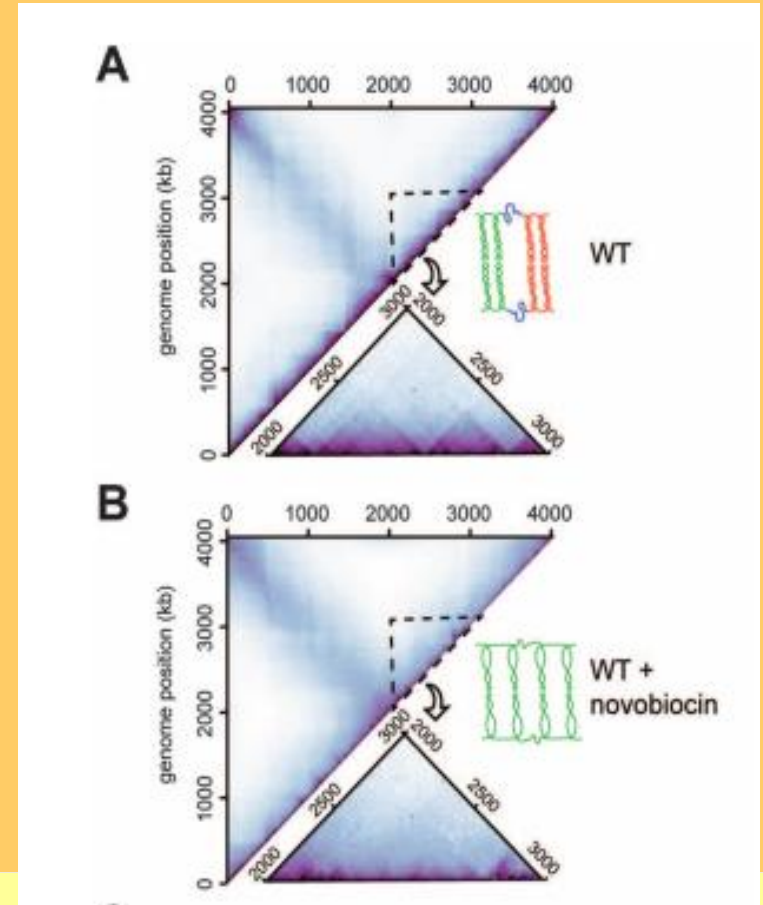
Simulation

❑ we increased the spacing between duplexes in a plectoneme monomer and increased the average spacing between plectonemes fivefold. Subsequent simulations reproduced the partial loss of CID boundaries and changes in contact frequencies .

❑ Demonstrating that supercoiling is critical to ge-

nome compaction in the 20- to 200-kb range and helps establish CIDs in vivo.

Part5 nucleoid-associated proteins

1.The interaction matrix for Δhup1Δhup2 was grossly similar to that of wild-type cells .
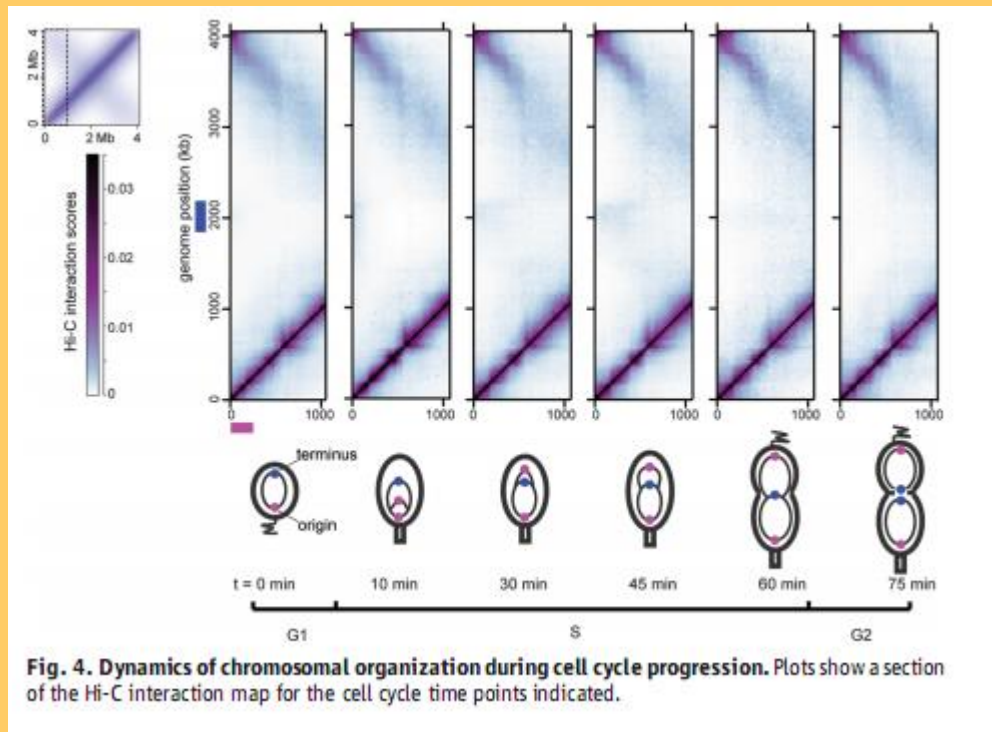2.The contact probability plot for Dhup1Dhup2 cells revealed a significant decrease in short-range contacts, up to ~100 kb.
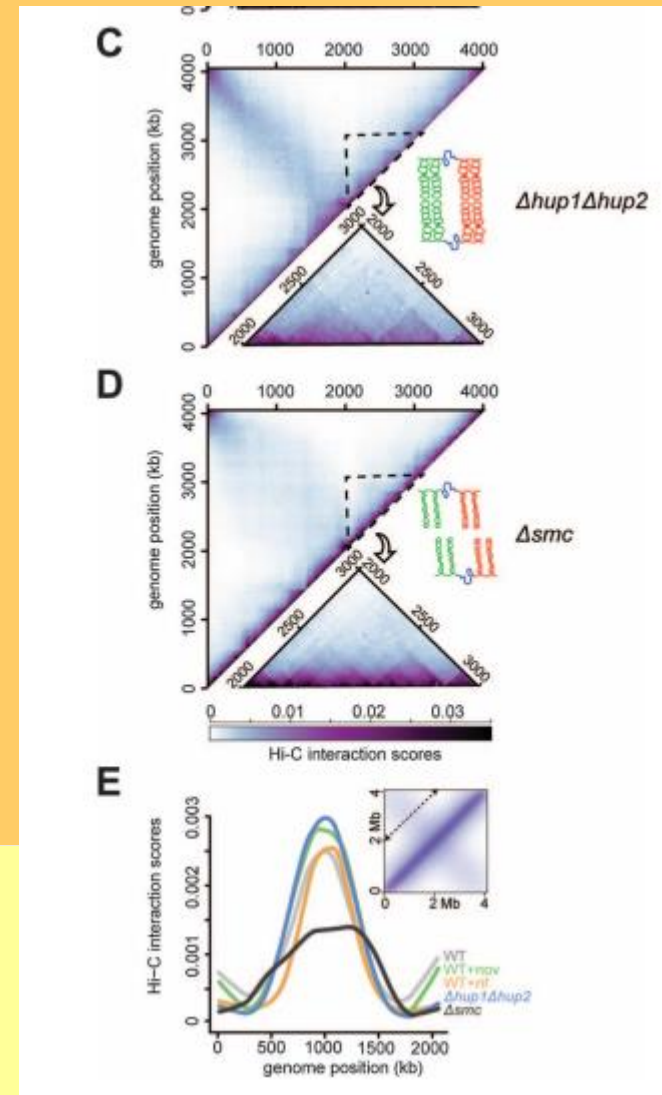
❑Hi-C analysis of Caulobacter Δsmc swarmer cell showed a clear drop in the frequency of inter-chromosomal arm interactions.

❑ the frequencies of intra-arm interactions and CID boundaries were largely unaffected.

Δ Our data suggest that Caulobacter SMC contributes primarily to the colinearity of chromosome arms.

Part6
chromosome organization changes

Fig. 4. Dynamics of chromosomal organization during cell cycle progression. Plots show a section of the Hi-C interaction map for the cell cycle time points indicated.

1.As the cell cycle progressed, the Hi-C contact maps indicated progressively more interactions between origin and terminus-proximal loci.
2. The frequency of origin and terminus didn't change.
3. The CIDs identified in swarmer cells remained intact throughout the cell cycle.

❑ This implies that the translocating chromosome is largely insulated from the anchored chromosome despite their physical proximity.

❑ CIDs must get reestablished concurrently with, or shortly after, DNA replication.

Conclusions

❑ Domains on a 100-kb length scale are a fundamental unit of chromosome structure in all organisms.

❑Caulobacter CIDs often appear as nested domains.

❑ Domain barriers in Caulobacter are relatively fixed; however, within each CID there could be additional barriers that arise and dissipate.

❑ DNA binding proteins HU and SMC contribute to chromosome organization, they do not significantly affect CIDs.

❑Supercoiling and highly expressed genes are critical determinants of domain formation in bacteria,and we suspect that similar mechanisms contribute to creating TADs in higher organisms.

THANK YOU !